R. Banks, J. Becker, P. Cannell, F. Gill, S. Lanyon, S. Olson, K. Parkes and J. Pitocchelli reviewed the manuscript.

LITERATURE CITED

AMERICAN ORNITHOLOGISTS' UNION. 1983. Check-list of North American birds, 6th ed. Amer. Ornithol. Union, Washington, D.C.

AVISE, J. C., J. C. PATTON, AND C. F. AQUADRO. 1980. Evolutionary genetics of birds. Comparative molecular evolution in New World warblers and rodents. J. Heredity 71: 303–310.

BALDWIN, S. P., H. C. OBERHOLSER, AND L. G. WORLEY. 1931. Measurements of birds. Cleveland Mus. Nat. Hist. Vol. 2.

BREWSTER, W. 1874. A new species of North American warbler. Am. Sportsman 5:33.

———. 1881. On the relationship of *Helminthophaga leucobronchialis,* Brewster, and *Helminthophaga Lawrencei,* Herrick; with some conjectures respecting certain other North American birds. Bull. Nuttall Ornith. Club 6:218–225.

GILL, F. B. 1980. Historical aspects of hybridization between Blue-winged and Golden-winged warblers. Auk 97:1–18.

HERRICK, H. 1875. Description of a new species of *Helminthophaga.* Proc. Acad. Sci. Philadelphia 26 (1874):220.

LANGDON, F. W. 1880. Description of a new warbler of the genus *Helminthophaga.* J. Cincinnati Soc. Nat. Hist. 3:119–120.

MCCAMEY, F. 1950. A puzzling hybrid warbler from Michigan. Jack Pine Warbler 28: 67–72.

PARKES, K. C. 1951. The genetics of the Golden-winged × Blue-winged Warbler complex. Wilson Bull. 63:5–15.

———. 1978. Still another parulid intergeneric hybrid (*Mniotilta* × *Dendroica*) and its taxonomic and evolutionary implications. Auk 95:682–690.

RIDGWAY, R. 1880. Note on *Helminthophaga cincinnatiensis,* Langdon. Bull. Nuttall Ornith. Club 5:237–238.

———. 1885. *Helminthophaga leucobronchialis.* Auk 2:359–363.

SHORT, L. L. JR. AND C. S. ROBBINS. 1967. An intergeneric hybrid wood warbler (*Seiurus* × *Dendroica*). Auk 84:534–543.

GARY R. GRAVES, *Dept. Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560.* *Received 17 July 1987, accepted 2 Dec. 1987.*

Wilson Bull., 100(2), 1988, pp. 289–293

Techniques for study of avian syringes.—In most birds, the syrinx is located within the thoracic cavity, dorsal to the heart and ventral to the esophagus (in hummingbirds and some storks it is along the neck). The syrinx and trachea are bound to the esophagus by membrane; removal of these together, using the esophagus as a 'handle,' reduces risk of damage to the delicate bronchi and preserves membraneous connections (e.g., the bronchidesmus). Removal of a syrinx from a museum specimen disrupts the heart arteries and the sternum and breast muscles along one side, but careful removal leaves a specimen largely intact and makes the syrinx available for future researchers. Dissection procedures described here follow Ames (1971:13) but are more detailed:

(1) With the bird on its back, expose the skin along the neck and chest along the bird's right side.

(2) With scissors, cut shallowly through the skin of the neck until the trachea is found, usually to the right of the vertebral column. Initial cuts higher along the neck minimize damage to the trachea. Expose the trachea to its entry into the thoracic cavity at the furculum.

(3) Cut the furculum and the cephal edge of the sternum just to the right of the midline. Continue this cut, straight and *shallow,* through the right sternum and breast musculature; deep cuts may damage the heart or syrinx. In some birds, e.g., some owls and goatsuckers, the syrinx may be removed through the furcular opening without damaging the skeleton. The syrinx can be removed from skeletal preparations by working through openings between the ribs or through the abdominal cavity after the viscera have been removed.

(4) Open the thoracic cavity enough to see both M. sternotrachealis where they extend from the trachea toward the thoracic wall. Note their precise position of insertion, then cut through each muscle near to the thoracic attachment.

(5) Cut through the trachea and esophagus (or just the trachea if the esophagus is not being removed) far enough above the syrinx to include all 'syringeal' features and to provide a handle for manipulation of the specimen. It is better to include too much trachea than too little. Cutting away connecting tissue, free the trachea and esophagus (or just the trachea) down to the level of the heart.

(6) Carefully cut through the major heart arteries, allowing the heart to be lifted up to the left, exposing the syrinx and bronchi. With curved-blade scissors, cut through the right bronchus where it enters the lung, the esophagus cephal to the syrinx (if the esophagus is also to be removed), and the left bronchus where it enters the lung. Include as much of the bronchi as possible; short bronchi may be the most common deficiency of syrinx specimens.

(7) Using forceps, pull *gently* on the esophagus and continue to cut away attached connective tissue from around the trachea, syrinx, bronchi, and esophagus until these can be removed *easily.* If the esophagus is to be left within the body, use forceps to pull on the trachea and cut the tissues between the syrinx and the esophagus as well as other restraining tissue. It may be advisable to bind the carcass closed with twine, thread, or cheesecloth before returning it to its storage jar.

(8) In most syringes, dorsal and ventral surfaces may be distinguished by the greater dorsal exposure of the internal tympaniform membranes and the ventral tendency of the paired M. sternotrachealis. If there is doubt (e.g., picid syringes approach dorsoventral symmetry), notch the caudal end of the trachea on the dorsal surface as a marker and note that this has been done.

(9) Place fresh syringes in buffered 10% formalin for several days (see Cato 1986:26). Blot excess formalin, and transfer to 70%–75% ethanol (ETOH) for storage. Syringes from fixed museum specimens may be put directly into alcohol. For long-term storage, small alcohol-filled vials including the syrinx and appropriate labelling should be placed within larger alcohol-filled containers. In the field, *small* labels bearing field catalogue numbers may be tied with cotton thread gently but securely around attached tracheae and esophagi and placed with other syringes in a single container.

(10) The specimen may be 'cleaned' at your convenience. Artifacts within the respiratory tract can usually be removed by squirting alcohol through the trachea with a syringe. Care must be used in cleaning; removal of connective tissue may expose important features but at the cost of others, including embedded muscle or nerves.

Osteological preparation.—Normally, the syrinx and trachea are removed from skeletal preparations and discarded. If included, delicate articulations usually are broken by larger bones within crowded skeleton boxes. Since most adult birds have ossified trachea and virtually all have ossified syringes, it is useful and appropriate to preserve the syrinx as a

skeletal element during the preparation of skeletal specimens. First, before removing the viscera from the body cavity, cut through the bronchi, esophagus, and associated membranes at the level of the lungs, leaving them attached to the dorsal body wall of the thorax. Second, submit the specimens to minimal dermestid activity (terrestrial isopods may be preferable in preparation of delicate skeletal material, Maiorana and Van Valen 1985). Third, store the syrinx and other delicate skeletal elements in a smaller box or envelope within the primary skeleton box.

Skeletal syrinx specimens lack muscles and membranes. Their cartilaginous components may change shape with drying. However, they are more convenient for some measurements and for S.E.M. photography. In addition, because of scarcity of whole alcoholic specimens (Zusi et al. 1982), skeletal materials may provide the only syringeal source for many species.

Muscle staining.—The myology of many syringes is simple, consisting of only two pairs of muscles, M. sternotrachealis and M. tracheolateralis. Other syringes, including those of Coliiformes, Trochilidae, Psittaciformes, and most Alcedinidae and Passeriformes, possess a more complex, intrinsic musculature (Cannell 1986). The number, length, and fiber direction of these muscles may be difficult to determine because they are often small, thin, or obscured by connective tissue. Examination may be facilitated by using an iodine stain to darken fibers. By immersing specimens in a solution of distilled water (100 ml), potassium iodide (2.0 g), and iodine (1.0 g) for several minutes, muscles become a dark reddish-brown (Bock and Shear 1972). This stain, fading under light, is reversible and nondestructive; hence, iodine-stained specimens may undergo subsequent staining procedures. The stock solution should be stored away from light.

Clearing and staining for cartilage and bone.—This procedure distinguishes between cartilage and bone and clarifies the configuration of all supporting structures. Hence, perception of syringeal structure is greatly improved. Here, procedures of Wassersug (1976) and Dingerkus and Uhler (1977) have been adapted specifically for use on syringes, but a similar treatment could be used on avian embryos or other small materials. Note that the process is destructive, obliterating details of muscle and membrane and potentially altering the overall configuration. Rare specimens should only be cleared and stained after important myological and membranous features have been recorded in detail. Even for common species, muscles and membranes should be examined and measurements of syringeal parts made before clearing and staining. No syrinx should be cleared or stained or submitted to any other destructive procedure without permission from the host institution. Pre-1940 museum specimens may not have been fixed; syringes from these should be placed in formalin for several days. These and specimens stored in isopropyl alcohol should be kept in ETOH for a week before initiating staining.

This procedure takes at least 4 days, but may be interrupted when materials are in water or alcohol, or by refrigeration during the enzyme step. Large syringes may require longer periods at some steps. Other factors, such as quality of fixation and preservation, will also influence results. Excess tissue, clotted blood, and the esophagus should be removed prior to staining. These directions make solutions of 150 ml, adequate for staining ten small-to-medium syringes in individual 20 ml scintillation vials.

(1) Stain cartilage with alcian blue for 24 h (no danger of overstaining). *Alcian blue stain:* 30 ml glacial acetic acid; 120 ml 95% ETOH; 20 mg alcian blue powder. Color should not be so dense as to obscure position of specimen. Solution is unstable and *must be made up fresh.* This is a tenacious stain that should be handled carefully.

(2) Rinse in 95% ETOH for several minutes (until specimen sinks). This solution is reusable.

(3) Rinse in 50% ETOH for several minutes (until specimen sinks). This solution is reusable.

(4) Rinse in distilled water.

(5) Clear in enzyme solution for 5–6 h, or longer, until membranous parts are clear. Large or tough specimens may need to be cleared for several days. Specimens retaining blue stain after a day or so in enzyme solution may be put in 1% KOH for several hours; KOH and enzyme solutions may be alternated until membranous parts are clear. Note that KOH destroys myelin, and its use will inhibit nerve staining. This solution is temperature dependent; activity can be increased by mild heating (27°C is ideal) or virtually halted with refrigeration. *Enzyme solution:* 40 ml saturated aqueous sodium borate (distilled water saturated with borax); 110 ml distilled water; 2 g (½ teaspoon) trypsin. Stir gently until dissolved. This solution *must be made up fresh.*

(6) Rinse gently in tap water (but not over open sink drain).

(7) Stain for calcium phosphates with alizarin red S for 12–14 h (no danger of overstaining up to 24 h). *Alizarin red stain solution:* 10 ml 10% KOH stock solution (10 g of pellets per 100 ml distilled water); 190 ml distilled water; enough alizarin red S powder to color solution deep purple. Reusable until solution loses color or effectiveness is reduced.

(8) Rinse gently in tap water, then dehydrate through a series of KOH/glycerin solutions (a to c, below). These are stable and may be reused until they become too colored. Leave syringes in each step for 5 or more hours.

a. 3:1 KOH/glycerin: 30 ml 10% KOH; 270 ml distilled water; 3 ml 3% hydrogen peroxide (bleaches pigments); 100 ml glycerin.

b. 1:1 KOH/glycerin: 30 ml 10% KOH; 270 ml distilled water; 3 ml 3% hydrogen peroxide; 300 ml glycerin.

c. 1:3 KOH/glycerin: 15 ml 10% KOH; 135 ml distilled water; 450 ml glycerin.

(9) Store syringes in solution of: 90% glycerin, 10% distilled water; a few thymol crystals (inhibits mold and bacteria).

If results are unsatisfactory, specimens may be recleared or restained with alizarin red, but once in glycerin, specimens should be rehydrated back through the glycerin series (1:3, 1:1, 3:1) and washed in distilled water before further treatment; glycerin negates enzyme activity and may affect stain effectiveness. It is possible to restain for alcian blue but there is a chance of losing the specimen (G. Dingerkus, pers. comm.).

Nerve staining.—This procedure is adopted from Filipski and Wilson (1984, 1985), A. Savitsky (pers. comm.), and S. Gornak (unpubl.). It can be used independently, with the cartilage and bone staining procedure, or applied to previously cleared and stained specimens. Material to be stained for nerves alone should first be cleared with trypsin (see step 5 above), then washed in distilled water. For integration within the cartilage and bone staining procedure, insert steps described below between steps 6 and 7 above. Specimens previously cleared and stained should be rehydrated, soaked in distilled water for several hours, and then submitted to the following procedure.

Three provisos accompany this technique. First, nerves removed during the initial 'cleaning' can no longer be stained. Second, KOH is destructive to myelin, so specimens treated with KOH may not provide effective nerve staining. Third, nerve staining may not be permanent; observed patterns should be recorded while still visible. Nerves can be restained but possibly with reduced effectiveness (rehydrate specimens before restaining).

(1) Immerse in 70–75% ETOH for 30 min or more.

(2) Immerse in Sudan Black B stain. Check frequently at first, then every 5–10 minutes; remove when nerves are a dense blue-black. Gentle agitation facilitates staining. *Sudan Black B stain*: dissolve 0.5 g Sudan Black B in 500 ml of 70% ETOH with the aid of a warm water bath. Filter solution before use.

(3) Destain in 70–75% ETOH for 5 min or more, then rinse gently in tap water. (If staining for calcium, go to step 7 of clearing and staining procedure above.)

(4) Dehydrate through a KOH/glycerin series as in step 8 above.

(5) Transfer to 90% glycerin as in step 9 above.

Acknowledgments.—I thank A. Savitsky for information about nerve staining. Comments from C. Blem, M. Clench, C. Conway, T. Crowe, G. Dingerkus, W. Lanyon, and R. Zusi helped improve the manuscript; particular thanks are due W. Lanyon.

LITERATURE CITED

AMES, P. L. 1971. The morphology of the syrinx in passerine birds. Peabody Mus. Nat. Hist., Yale Univ. Bull. No. 37:1–194.

BOCK, W. J. AND C. R. SHEAR. 1972. A staining method for gross dissection of vertebrate muscles. Anat. Anz. 130:222–227.

CANNELL, P. F. 1986. Syringeal complexity and the ordinal relationships of "higher" birds. Ph.D. diss., City University of New York, New York.

CATO, P. S. 1986. Guidelines for managing bird collections. Texas Tech Press, Lubbock, Texas.

DINGERKUS, G. AND L. D. UHLER. 1977. Enzyme clearing of alcian blue stained whole vertebrates for demonstration of cartilage. Stain Technol. 52:229–232.

FILIPSKI, G. T. AND M. V. H. WILSON. 1984. Sudan Black B as a nerve stain for whole cleared fishes. Copeia 1984:204–208.

——— AND ———. 1985. Staining nerves in whole cleared amphibians and reptiles using Sudan Black B. Copeia 1985:500–502.

MAIORANA, V. C. AND L. M. VAN VALEN. 1985. Terrestrial isopods for preparing delicate vertebrate skeletons. Syst. Zool. 34:242–245.

WASSERSUG, R. J. 1976. A procedure for differential staining of cartilage and bone in whole formalin-fixed vertebrates. Stain Technol. 51:131–134.

ZUSI, R. L., D. S. WOOD, AND M. A. JENKINSON. 1982. Remarks on a world-wide inventory of avian anatomical specimens. Auk 99:740–757.

PETER F. CANNELL, *Division of Birds, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560.* *Received 16 June 1987, accepted 3 Nov. 1987.*

Wilson Bull., 100(2), 1988, pp. 293–294

Avian Davian Behavior.—Dickerman (J. Mamm. 41:403, 1960) reported a field observation of a male Richardson ground squirrel (*Citellus* [=*Spermophilus*] *richardsoni*) copulating with a dead female which was lying on its side in a copulatory position. He termed the behavior "Davian behavior," a facetious reference to a ribald Limerick about necrophilia. Here I make what is apparently the first report of Davian behavior in birds.

I observed several drake Mallards (*Anas platyrhynchos*) attempting forced copulation with a hen Mallard at 16:00 on 9 April 1987 on Sheldon Lake, Fort Collins, Colorado. After several minutes of constant pursuit, the hen was forced to swim to the shoreline of an island. She stopped at the water's edge, apparently exhausted and unable to escape farther. She was immediately approached by two Chinese Geese (*Anser cygnoides*), one of which repelled the drakes to a distance of approximately 2 m. It then stood over the hen in a pre-copulatory position and began to peck violently at the back of her head and neck. After approximately 5 min the hen became motionless in a copulatory posture, and she was not observed to move again; she was apparently dead at this point. The goose continued to peck for another